REMARKS

The Invention

The present invention relates to methods for increasing the efficiency of the transfection of cells. The methods involve first administering a cell cycle blocking agent to a patient and second administering a nucleic acid to the patient. The nucleic acid is administered in a lipid formulation comprising a cationic lipid and a lipid derivative selected from an ATTA-lipid, a polyethylene glycol (PEG)-lipid derivative, and a ganglioside G_{M1}-modified lipid and the nucleic acid is fully encapsulated in the lipid formulation such that less than 5% of the nucleic acid is degraded after exposure of the formulation to 1 U DNAse I for 30 minutes in digestion buffer at 37°C. The invention further relates to cancer therapy and, in particular, to methods of efficiently transfecting cancer cells with nucleic acids.

Status of the Claims

Applicants wish to thank Examiner Woitach for extending the courtesy of the telephonic interview held on August 12, 2003 with Applicants' representatives Carol A. Fang and Eugenia Garrett-Wackowski. During this interview, a number of issues were clarified which have helped Applicants to more fully address the concerns of the Examiner. Applicants thank Examiner Woitach for his time.

After entry of this amendment, claims 38-44 and 46-48, 49-79, and 82-84 are pending. Claims 49 and 80-81 have been canceled without prejudice to future prosecution. Claims 38, 42, 69, 71-73, and 82 have been amended. More particularly, claims 38 and 69 have been amended to recite that the "nucleic acid is administered in a lipid formulation comprising a cationic lipid and a lipid derivative selected from the group consisting of an ATTA-lipid, a polyethylene glycol (PEG)-lipid derivative, and a ganglioside G_{M1}-modified lipid" and that the "nucleic acid is fully encapsulated in said lipid formulation such that less than 5% of the nucleic acid is degraded after exposure of said formulation to 1 U DNAse I for 30 minutes in digestion buffer at 37°C." Support for this amendment is found in the specification on page 27, lines 5-10,

which recites that stable plasmid-lipid particles can be formulated using the methods and compositions set out in U.S. Patent No. 5,705,385. The '385 patent was specifically incorporated by reference on page 27, line 10. The '385 patent describes the claimed methods of formulating the lipid-nucleic acid particles of claim 38 at, *inter alia*, col. 8, lines 52-64. Claim 42 has been amended to recite "Gene Directed Enzyme Prodrug Therapy ('GDEPT')." As explained previously, it was well known in the art at the time of filing that the acronym GDEPT stood for Gene Directed Enzyme Prodrug Therapy (see, e.g., Connors and Knox, Stem Cells 13(5):501-11 (1995), abstract and Connors, Gene Ther. 2(10): 702-9 (1995), abstract, copies previously submitted). Claims 71-73 have been amended to solely correct grammatical errors. Claim 82 has been amended solely to ensure correct dependency. Thus, no new matter is added by these amendments.

Entry of these amendments is respectfully requested. As discussed with the Examiner during the interview of August 12, 2003, these amendments incorporate recitations from dependent claims into the first independent claim. Therefore, the amendments encompass embodiments of the invention already in the claims as previously searched. Thus, these amendments raise no new issues that would require additional consideration or searching.

A clean copy of the claims is provided in Appendix A for the Examiner's convenience.

Objection to claim 42

Claim 42 has been objected to for the recitation "GDEPT." In accordance with the Examiner's suggestion, claim 42 has been amended to recite "Gene Directed Enzyme Prodrug Therapy ("GDEPT")."

Rejection Under 35 U.S.C. § 102(b)

Claims 38-44, 47-50, 69-73, 79-81, and 84-86 were initially rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Son *et al.*, *Proc. Natl. Acad. Sci.* (1994), 91:12669-12672.

As explained previously, for a rejection of claims under § 102(b) to be properly founded, the Examiner must establish that a single prior art reference discloses each and every element of the claimed invention. *See, e.g., Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 U.S.P.Q. 81 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987). In *Scripps Clinic & Research Found. v. Genentech, Inc.*, 18 U.S.P.Q.2d 1001 (Fed. Cir. 1991), the Federal Circuit held:

> [A]nticipation requires that all of the elements and limitations of the claim are found with a single prior art reference. . . .There must be ***no difference*** between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention.

Id. at 1010 (emphasis added). Anticipation can be found, therefore, only when a cited reference discloses ***all*** of the elements, features or limitations of the presently claimed invention.

The present claims are directed to methods for introducing a nucleic acid encoding a foreign gene into cells in a patient by first administering a cell cycle blocking agent to the patient and second administering the nucleic acid to the patient, whereby transfection efficiency is increased by at least 50%. The nucleic acid is administered in a lipid formulation comprising a cationic lipid and a lipid derivative, and the nucleic acid is fully encapsulated in the lipid formulation.

Son *et al.* is cited as teaching that cell cycle blocking agents can increase transfection efficiency by at least 50%. As discussed with the Examiner, Son *et al.* describes injection of a plasmid-liposome ***complex*** formed by mixing preformed liposomes with plasmid DNA. In contrast to the presently claimed methods, the plasmid DNA of Son *et al.* is not encapsulated in the liposome. During the interview, the Examiner agreed that administration of nucleic acids fully encapsulated in a lipid formulation is distinguishable from administration of plasmid-liposome ***complexes*** described in Son *et al.* Thus, an element of the invention is absent from the disclosure of Son *et al.* and Son *et al.* does not anticipate the claimed invention.

Moreover, as acknowledged by the Examiner during the interview, Son *et al.* disclose that only one cell cycle blocking agent, *i.e.*, cisplatin, was effective in sensitizing cells to transfection. Son *et al.* explicitly states that "only cisplatin could significantly sensitize the

tumor for *in situ* lipofection" (page 12671, right hand column). Son *et al.* also present data showing that only cisplatin sensitizes tumors for *in situ* lipofection (Fig. 4). Moreover, as discussed with the Examiner, Son *et al.* teach that ***several*** other anticancer drugs such as methotrexate, etoposide, cytosine arabinonucleoside, doxorubicin, and carboplatin (a geometric isomer of cisplatin) ***had no effect*** on transfection. The Examiner agreed that Son *et al.*'s own interpretation of the data presented in Figure 4 explicitly states that:

> Fig. 4 shows that only cisplatin could significantly sensitize the tumor for *in situ* lipofection. Other anticancer drugs including methotrexate, etoposide, cytosine arabinonucleoside, doxorubicin, and vincristine had no effect. Transplatin, a geometric isomer of cisplatin that has no anticancer activity also showed no effect.

Thus, as acknowledged by the Examiner, based on Son *et al.*'s own explicit statements, the cited reference does not disclose all of the elements of the present invention because, in contrast to the claimed invention, Son *et al.* teach that only cisplatin would be useful for methods of introducing a nucleic acid into cells in patient.

Thus, Son *et al.* fail to disclose all of the elements of the claimed methods of introducing a nucleic acid to cells in a patient using the cell cycle blocking agents recited in the claims (*i.e.*, cyclophosphamide, taxol, taxolene, and a vinca alkaloid), wherein the nucleic acid is administered in a lipid formulation comprising a cationic lipid and a lipid derivative, and the nucleic acid is fully encapsulated in the lipid formulation. Thus Son *et al.* do not anticipate the claimed invention. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. §102(b) be withdrawn.

Rejections Under 35 U.S.C. § 103(a)

The claims are rejected, in various combinations, under 35 U.S.C. § 103(a) over a number of different references. As explained previously, to establish a *prima facie* case of obviousness, (1) there must be some suggestion or motivation, either in the reference themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) there must be a reasonable expectation of success; and (3)

the prior art reference (or references when combined) must teach or suggest all the claim elements. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. (*See*, M.P.E.P., § 2143, citing *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

1. Rejection of claims 38-44, 47-50, 69-73, and 78-86 over Son *et al.* and Roth *et al.*

Claims 38-44, 47-50, 69-73, and 78-86 were initially rejected under 35 U.S.C. § 103(a) as unpatentable over Son *et al.*, and Roth *et al.* (U.S. Patent No. 5,747,469), and Walker *et al.* (U.S. Patent 6,041,252). In making the rejection, the Examiner alleges that Son *et al.* teach that other agents besides cisplatin increase transfection efficiency, that Walker *et al.* disclose general methods for improving the delivery of liposomal compositions and concludes that one of skill in the art would be motivated by Son *et al.* to use any delivery method conventional in the art.

As explained above, the present invention is directed to methods for introducing a nucleic acid encoding a foreign gene into cells in a patient by first administering a cell cycle blocking agent to the patient and second administering the nucleic acid to the patient, whereby transfection efficiency is increased by at least 50%. The nucleic acid is administered in a lipid formulation comprising a cationic lipid and a lipid derivative, and the nucleic acid is fully encapsulated in the lipid formulation.

As discussed in detail above in connection with the 35 U.S.C. § 102(b) rejection, Son *et al.* does not disclose all of the elements, features or limitations of the presently claimed invention because, in contrast to the present invention, Son *et al.* contains no disclosure of the claimed methods of introducing a nucleic acid to cells in a patient, wherein the nucleic acid is administered in a lipid formulation comprising a cationic lipid and a lipid derivative and the nucleic acid is fully encapsulated in the lipid formulation and ***teach away*** from the use of drugs other than cisplatin to enhance transfection efficiency.

For example, as explained above, and acknowledged by the Examiner during the interview, Son *et al.*, describes administration of plasmid-liposome ***complexes***, not

administration of a nucleic acid fully encapsulated in a lipid formulation as presently claimed. The Examiner also acknowledged that Son *et al.* explicitly states that ***only*** cisplatin significantly sensitizes tumor cells for transfection and that other anticancer drugs, including vincristine, have no effect on transfection efficiency. Son *et al.*' s interpretation of their own data explicitly states that: "Fig. 4 shows that only cisplatin could significantly sensitize the tumor for in situ lipofection. Other anticancer drugs including methotrexate, etoposide, cytosine arabinonucleoside, doxorubicin, and vincristine had no effect." As discussed with the Examiner, in view of the teachings of Son *et al.*, one of skill in the art at the time of the present invention would have had ***no motivation*** to use any drug except cisplatin to improve transfection efficiency. The Examiner acknowledged that based on the disclosure of Son *et al.*, one of skill in the art would not have been motivated to optimize the methods of Son *et al.* for any other drugs. Thus, if anything, Son *et al.* teach away from the use of the compounds recited in the claimed invention (*e.g.*, cyclophosphamide, taxol, taxolene, vinblastine, vincristine, vinorelbine, and a vinca alkaloid).

Roth *et al.* does not remedy the defect in Son *et al.* In contrast to the claimed invention, Roth *et al.* disclose contacting cells with agents such as cisplatin, doxorubicin, etoposide, verapamil, podophyllotoxin, and 5-fluorouracil (*see,* claims 4, 6, 8, 10, 11, and 12, respectively). Roth *et al.* thus fail to disclose the use of any of the cell cycle blocking agents recited in the claims of the present invention. Therefore, even if the teachings of Son *et al.* and Roth *et al.* were combined, the combination would not lead to the claimed invention because the references, either alone or in combination, fail to teach or suggest introducing a nucleic acid encoding a foreign gene into a cell in a patient by administering any of the cell cycle blocking agents (*e.g.*, cyclophosphamide, taxol, taxolene, vinblastine, vincristine, vinorelbine, and a vinca alkaloid), wherein the nucleic acid is administered in a lipid formulation comprising a cationic lipid and a lipid derivative, and the nucleic acid is fully encapsulated in said lipid formulation, as recited in the present claims.

Walker *et al.* do not cure the deficiency of Son *et al.* and Roth *et al.* Walker *et al.* disclose the use of electrical fields to deliver therapeutic agents encapsulated in a liposome.

Walker *et al.* contains no mention or suggestion of the use of any cell blocker or the introduction of a nucleic acid into a cell. Walker *et al.* does not even contain the words "nucleic acid." Therefore, one of skill in the art would not have had the motivation to combine Walker *et al.* with Son *et al.* and Roth *et al.* Even if one of skill in the art were to combine Son *et al.*, Roth *et al.*, and Walker *et al.*, the combination would not lead to the claimed method of introducing a nucleic acid into cells in a patient.

Absent a teaching or suggestion of a method of introducing a nucleic acid into cells in a patient using the cell cycle blocking agents recited in the claims (*i.e.*, cyclophosphamide, taxol, taxolene, and a vinca alkaloid), wherein the nucleic acid is administered in a lipid formulation comprising a cationic lipid and a lipid derivative and the nucleic acid is fully encapsulated in the lipid formulation, as disclosed and claimed in the present invention, the present invention is non-obvious, and thus, patentable. Accordingly, Applicants respectfully request withdrawal of this rejection under 35 U.S.C. § 103.

2. Rejection of claims 74-77 and 87 over Son *et al.*, Roth *et al.*, and Bally *et al.*

Claims 74-77 and 87 were initially rejected under 35 U.S.C. § 103(a) as unpatentable over Roth *et al.*, Son *et al.*, and Bally *et al.* (US Patent 5,705,385). In making this rejection, the Examiner alleges that Bally *et al.* teach general methods for improving gene delivery methods and that Son *et al.* provides motivation for one of skill in the art to optimize gene delivery protocols. Applicants respectfully traverse this rejection.

As discussed in detail above, Son *et al.* and Roth *et al.* alone or in combination do not disclose all of the elements, features or limitations of the presently claimed invention. In contrast to the present invention, Son *et al.* (1) contains no disclosure of the claimed methods of introducing a nucleic acid to cells in a patient, wherein the nucleic acid is administered in a lipid formulation comprising a cationic lipid and a lipid derivative and the nucleic acid is fully encapsulated in the lipid formulation, and (2) teaches away from the use of drugs other than cisplatin to enhance transfection efficiency. Roth *et al.* fail to disclose the use of any of the cell

cycle blocking agents (*e.g.*, cyclophosphamide, taxol, taxolene, vinblastine, vincristine, vinorelbine, and a vinca alkaloid) recited in the present claims. Moreover, as acknowledged by the Examiner, one of skill in the art would have ***no motivation*** to optimize the methods of Son *et al.* for any other drugs.

Bally *et al.* fail to cure the deficiency in Son *et al.* and Roth *et al.*. Bally *et al.* does not disclose the use of ***any*** claimed cell cycle blocking agents: cyclophosphamide, taxol, taxolene, and a vinca alkaloid. Moreover, there is no mention or suggestion in Bally *et al.* of the use of a cell cycle blocking agent. Therefore, one of skill in the art would not have had the motivation to combine Bally *et al.* with Son *et al.* and Roth *et al.* Even if one of skill in the art were to combine Son *et al.*, Roth *et al.*, and Bally, *et al.*, the combination would not lead to the claimed method of introducing a nucleic acid encoding a foreign gene into a cell in a patient.

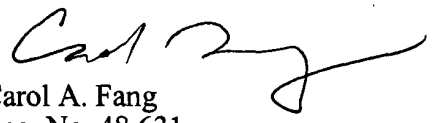
Absent a teaching or suggestion of a method of introducing a nucleic acid into cells in a patient using the cell cycle blocking agents recited in the claims (*i.e.*, cyclophosphamide, taxol, taxolene, and a vinca alkaloid), wherein the nucleic acid is administered in a lipid formulation comprising a cationic lipid and a lipid derivative and the nucleic acid is fully encapsulated in the lipid formulation, as disclosed and claimed in the present invention, the present invention is non-obvious, and thus, patentable. Accordingly, Applicants respectfully request withdrawal of this rejection under 35 U.S.C. § 103.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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APPENDIX A
PENDING CLAIMS

38. (Currently amended) A method of introducing a nucleic acid encoding a foreign gene into a cell in a patient, said method comprising the steps of

(a) first administering a cell cycle blocking agent to said patient, wherein said cell cycle blocking agent is a member selected from the group consisting of cyclophosphamide, taxol, taxolene, and a vinca alkaloid; and

(b) second administering said nucleic acid to said patient within seven days of step (a), wherein said nucleic acid is administered in a lipid formulation comprising a cationic lipid and a lipid derivative selected from the group consisting of an ATTA-lipid, a polyethylene glycol (PEG)-lipid derivative, and a ganglioside G_{M1}-modified lipid,

wherein said nucleic acid is fully encapsulated in said lipid formulation such that less than 5% of the nucleic acid is degraded after exposure of said formulation to 1 U DNAse I for 30 minutes in digestion buffer at 37°C, and

further wherein transfection efficiency is increased by at least 50%.

39. (Original) The method of claim 38 wherein step (b) is performed within 3 days of step (a)

40. (Original) The method of claim 38 wherein step (b) is performed within 24 hours of step (a).

41. (Previously presented) The method of claim 38 wherein said foreign gene is a plasmid.

42. (Currently amended) The method of claim 38 wherein said foreign gene comprises a gene selected from the group consisting of genes encoding a cytokine, apoptotic protein, tumor suppressor, heat shock protein, immunogenic antigen, proteinase inhibitor, anti-

angiogenic protein, suicide gene for use in Gene Directed Enzyme Prodrug Therapy ("GDEPT"), ribozyme, antisense nucleic acid, viral protein and a toxin.

43. (Previously presented) The method of claim 38 wherein said foreign gene is administered systemically.

44. (Previously presented) The method of claim 38 wherein said foreign gene is administered locally or regionally.

47. (Previously presented) The method of claim 38, wherein said cell cycle blocking agent is selected from the group consisting of cyclophosphamide, taxol, and vincristine.

48. (Original) The method of claim 38 wherein said cell cycle blocking agent is in a liposome formulation.

49. (Canceled)

50. (Previously presented) A method of claim 38 wherein said cell cycle blocking agent is administered at least 32 h prior to administering said foreign gene.

51. (Previously presented) A method of claim 38 wherein said cell cycle blocking agent is administered at least 48 h prior to administering said foreign gene.

69. (Currently amended) A method of inhibiting growth of cancer cells in a patient having a cancer comprising introducing a nucleic acid encoding a foreign gene into a cell in a patient having cancer, said method comprising the steps of:

(a) first administering a cell cycle blocking agent to said patient, wherein said cell cycle blocking agent is a member selected from the group consisting of cyclophosphamide, taxol, taxolene, and a vinca alkaloid; and

(b) second administering said nucleic acid to said patient within seven days of step (a), wherein said nucleic acid is administered systemically in a lipid formulation comprising

a cationic lipid and a lipid derivative selected from the group consisting of an ATTA-lipid, a polyethylene glycol (PEG)-lipid derivative, and a ganglioside G_{M1}-modified lipid,

wherein said nucleic acid is fully encapsulated in said lipid formulation such that less than 5% of the nucleic acid is degraded after exposure of said formulation to 1 U DNAse I for 30 minutes in digestion buffer at 37°C, and

further,-wherein transfection efficiency is increased by at least 50%.

70. (Original) The method of claim 69, wherein said cancer comprises a tumor.

71. (Currently amended) The method of claim 70, wherein said cell cycle blocking agent and said foreign gene is administered distal to the site of the tumor.

72. (Currently amended) The method of claim 69, wherein said cell cycle blocking agent or said foreign gene is administered intravenously.

73. (Currently amended) The method of claim 69, wherein said cell cycle blocking agent or said foreign gene is administered intraperitoneally.

74. (Previously presented) A method of treating a patient having a cancer comprising introducing a nucleic acid encoding a foreign gene into a cell in said patient, said method comprising the steps of:

(a) first administering a cell cycle blocking agent to said patient, wherein said cell cycle blocking agent is a member selected from the group consisting of cyclophosphamide, taxol, taxolene, and a vinca alkaloid; and

(b) second administering said nucleic acid to said patient within seven days of step (a), wherein said nucleic acid is administered in a lipid formulation comprising a cationic lipid and a lipid derivative selected from the group consisting of an ATTA-lipid, a polyethylene glycol (PEG)-lipid derivative, and a ganglioside G_{M1}-modified lipid,

wherein said nucleic acid is fully encapsulated in said lipid formulation such that less than 5% of the nucleic acid is degraded after exposure of said formulation to 1 U DNAse I for 30 minutes in digestion buffer at 37°C, and

further wherein transfection efficiency is increased by at least 50%.

75. (Original) The method of claim 74, wherein said (PEG)-lipid derivative is a PEG-ceramide.

76. (Original) The method of claim 75, wherein said PEG-ceramide is a member selected from the group of PEG-Cer-C14, PEG-Cer-C20, and PEG-Cer-C8.

77. (Previously presented) The method of claim 74, wherein said lipid derivative is present in an amount of from about 1% to about 20% by weight of the lipid formulation.

78. (Original) The method of claim 74, wherein said lipid formulation is prepared by the method comprising:

(a) contacting said nucleic acid with a solution comprising non-cationic lipids and a detergent to form a nucleic acid-lipid mixture;

(b) contacting said cationic lipid with the nucleic acid-lipid mixture, thereby forming a charge-neutralized mixture of nucleic acids and lipids; and

(c) removing the detergent from the charge-neutralized mixture to provide the lipid-nucleic acid particles in which the nucleic acids are protected from degradation.

79. (Original) The method of claim 38, wherein the vinca alkaloid is a member selected from the group consisting of vinblastine, vincristine, and vinorelbine.

82. (Currently amended) The method of claim 38, wherein said lipid formulation is prepared by the method comprising:

(a) contacting said nucleic acid with a solution comprising non-cationic lipids and a detergent to form a nucleic acid-lipid mixture;

(b) contacting cationic lipids with the nucleic acid-lipid mixture, thereby forming a charge-neutralized mixture of nucleic acids and lipids; and

(c) removing the detergent from the charge-neutralized mixture to provide the lipid-nucleic acid particles in which the nucleic acids are protected from degradation.

83. (As filed) The method of claim 69, wherein the vinca alkaloid is a member selected from the group consisting of vinblastine, vincristine, and vinorelbine.

84. (As filed) The method of claim 74, wherein the vinca alkaloid is a member selected from the group consisting of vinblastine, vincristine, and vinorelbine.

85. (Original) The method of claim 38, wherein the foreign gene is a therapeutic gene.

86. (Original) The method of claim 69, wherein the foreign gene is a therapeutic gene.

87. (Original) The method of claim 74, wherein the foreign gene is a therapeutic gene.